(C) at least one visual means for visually differentiating host cells in different activation states of said readout system.

66. (**Amended**) A ~~K~~kit according to claim 65, wherein said host cells are bacterial cells or mammalian cells.

REMARKS

Claims 1, 2, 4-64, and 67-84 constitute the pending claims in the present application. Among them, claims 60-62 and 67 are withdrawn from further consideration as being directed to non-elected inventions or non-elected species. Applicants will cancel claims directed to non-elected inventions upon indication of allowable subject matter. Applicants have canceled claim 3 solely for the purpose of expediting prosecution. Applicants reserve the right to prosecute claims of identical or similar scopes in future applications.

Applicants note that the Examiner now considers amended claims 60, 62, and 67, formerly belonging to Group II, as part of the elected Group I. However, the Office Action asserts that these amended claims are directed to a non-elected species of the Group I invention. In addition, the Office Action asserts that amended claim 61 still belongs to the non-elected Group II as being a product-by-process claim that does not recite the method steps of claim 1. Accordingly, Applicants have amended claim 61 so that it now recites the method steps of claim 1, and thus should also be considered part of the elected Group I. Furthermore, Applicants respectfully traverse this restriction requirement on the ground that these amended claims depend on Group I claims, as the Examiner admits, and searching all Group I claims simultaneously will not impose serious burden on the Examiner. Finally, pursuant to MPEP 809, Applicants submit that if the independent claim 1 of Group I is allowable, all its dependent claims including claims 60-62 and 67 must be considered even if it requires withdrawal of the restriction requirement.

In addition, Applicants note that the Examiner has acknowledged that the following species, namely protein-protein, protein-peptide, and peptide-peptide, are closely related such that they all are included within the elected species from claim 4.

Applicants have also amended claims 1, 2, 4-6, 12, 17, 19, 20, 25, 26, 31, 32, 34-39, 43, 45-50, 53, 58, 59, 61, and 63-66, to correct obvious typographical or grammatical errors, or to clarify the subject matter being claimed. Applicants submit that there is no narrowing in scope due to any of these amendments. Support for these amendments can be found throughout the specification. For example, support for amendment to claim 34 can be found on page 35, last paragraph to page 36, first paragraph; support for amendments to claims 38 and 39 can be found on page 129, last paragraph, to page 130, first paragraph. Applicants have added new claims 68-82 to accommodate the amendment to claims 17, 35-37, 46, and 59.

Applicants have also added new kit claims 83-85. Support can be found throughout the specification, as well as in the originally filed claim 2.

The Office Action points out that the Oath or Declaration is defective because non-initialed alterations have been made, and the Examiner requests correction of this defect. Applicants have submit herewith a new Oath or Declaration in compliance with 37 C.F.R. 1.67(a) to comply with this requirement.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Claim rejections under 35 U.S.C. 112, first paragraph

Claims 1-59 and 63-66 are rejected under 35 U.S.C. 112, first paragraph, because the specification allegedly does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make/use the invention commensurate in scope with these claims.

Particularly, the Office Action argues that the instant claims lack any limitation regarding the genetic elements in the claims, or proteins encoded by these genetic elements, except for the positive limitation regarding activating the readout system upon interaction of the pair or complex of interacting molecules. Based on that argument, the Office Action concludes that some kind of limitations must be present - either in the genetic elements, the host cells, or the readout system - in order to differentiate the instant invention from the four types of false positives mentioned in page 3 of the specification. To support that point of view, the Office Action suggests that "if any

of the genetic elements contains activating domain(s) for the readout system, it would itself give a positive result without actual interaction of a pair or complex as desired in lines 1-2 of claim 1."

The specification describes four classes of potentially undesired hits that can result from a typical two-hybrid screen. Applicants wish to review the following terms and their definitions as used in the specification and this reply.

A typical conventional two-hybrid system described in the background section of the instant specification usually contains two hybrid proteins: a "bait hybrid" and a "fish hybrid." In an exemplary embodiment, the "bait hybrid" is a fusion protein of a "bait protein" and a DNA-binding domain. The "fish hybrid" corresponding to this embodiment would be a fusion protein of a "fish protein" and a transcription activation domain. In certain embodiments, the fish proteins are encoded by a library, while the bait protein is user-selected. However, in a library-library screen, both the bait and fish proteins are encoded by libraries. In other embodiments, the bait protein can be fused to the transcription activation domain, and the fish protein can be fused to the DNA-binding domain. The following passages assume that the bait protein is fused to a DNA-binding domain, although other embodiments are possible and are contemplated by the specification as filed.

As described in the specification on page 3, Class 1 errors / false positives result from a "bait protein" having a transcription activation domain of its own, and thus activation of the readout system is not dependent on interaction of the bait and the fish proteins (only the bait protein / DNA-binding domain fusion is needed for readout activation); Class 2 errors / false positives result from a "fish protein" having a DNA-binding domain and thus activation of the readout system is not dependent on interaction of the bait and the fish proteins (only the fish protein / transcription activation domain fusion is needed for readout activation); Class 3 errors / false positives result from the unintended interaction between the bait protein and the transcription activation domain on the fish hybrid, or interaction between the fish protein and the DNA-binding domain on the bait hybrid, thus activation of the readout system is not dependent on interaction of the bait and the fish proteins (but both fusions are still needed); Class 4 errors result from at least one of the bait and fish proteins (usually the bait protein) being a so-called "sticky protein," such

that activation of the readout system is not dependent on a *specific* interaction of the bait and the fish proteins (but both fusions are still needed).

First of all, based on the above description, classes 1-3 are clearly "false positives" by definition, while class 4, by definition, is not "false positive." Although the interaction between the bait and the fish proteins are due to the "stickiness" of at least one of the proteins, the interaction is nevertheless a *bona fide* interaction. The stickiness will not be apparent unless many interactions are identified, and even so, it is not conclusive evidence that the protein in question is "sticky." Nor can one conclude with confidence that the observed stickiness is of no biological or physiological significance. In fact, stickiness might be the nature of certain proteins, such as the heat shock proteins, that usually bind relatively non-specifically to many different proteins. In certain situations, it may even be desirable to identify interactions involving sticky proteins. Therefore, even if the claimed method may identify sticky proteins, it does not constitute a non-enabling embodiment of the invention.

Secondly, it is clear from the above discussion that all three classes of false positives do not involve interaction between the bait and the fish proteins. In the contrary, claims 1 and 2 are methods for identifying interacting molecules by employing various pre-selection steps (step (B) of claims 1 and 2) to eliminate those false positives before allowing an interaction to occur, so that host cells containing truly interacting proteins can be identified "upon activation of the readout system." (step (E) of claims 1 and 2). Therefore, to identify true positives, the method does involve interaction between the bait and the fish proteins (potentially interacting molecules).

The Office Action asserts that the contents of the genetic elements can vary widely thus unpredictably giving a readout system indication without requiring interaction of cloned proteins, etc. In support of that, the Office Action suggests that "if any of the genetic elements contains activating domain(s) for the readout system, it would itself give a positive result without actual interaction of a pair or complex as desired in lines 1-2 of claim 1." Thus, the Office Action alleges that (the specification) "lacks predictable enablement."

Applicants submit that such allegation is unfounded. In the above scenario, the result will be a class 1 false positive and thus, by definition, does not involve actual interaction between the bait and the fish proteins to activate the readout system. Therefore, such an embodiment will be

selected against in step (B), and thus will not be identified by any of the claimed methods. In addition, Applicants submit that the specification is enabled to its full scope by providing detailed descriptions of such pre-selection steps using host cells containing said genetic elements and readout systems.

Therefore, reconsideration and withdrawal of rejection under 35 U.S.C. 112, first paragraph is respectfully requested.

Claim rejections under 35 U.S.C. 112, second paragraph

Claims 1-59 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Office Action asserts that in claim 1, line 5, a plurality of genetic elements is recited, whereas as few as a single element practice of lines 3-4 conflict in number with the remainder of the claim. Accordingly, Applicants have amended claims 1-3, 5, and 47, to obviate this rejection. Applicants submit that there is no narrowing of scope due to these amendments.

The Office Action also asserts that in claim 1, line 2, the term "pool" is cited but nowhere in the claim steps can "pool" be found.

Applicants submit that the preamble normally does not carry any weight in determining the scope of a claim unless it is "'necessary to give life, meaning, and validity' to the claim" (MPEP 2111.02). Nevertheless, for clarification, Applicants have amended claims 1-3 to obviate this rejection. Applicants submit that there is no narrowing of scope in any respect due to these amendments.

The Office Action also suggests that Applicants remove indefinite claim languages such as "preferably." Accordingly, Applicants have amended claims 17, 35-37, and 59, and added new claims 68-82 to accommodate these amendments. Applicants submit that there is no narrowing of scope due to these amendments.

The Office Action further asserts that "automated" in claim 25, line 2 is unclear as to which subject it intends to modify. Therefore, Applicants have amended claims 19, 25, 31, 39, 43,

45, and 53 to clarify the issue. Applicants submit that there is no narrowing of scope due to these amendments.

Based on the above arguments, Applicants submit that all claims as amended comply with the requirement of 35 U.S.C. 112, second paragraph. Therefore, reconsideration and withdrawal of rejections under 35 U.S.C. 112, second paragraph is respectfully requested.

Claim rejections under 35 U.S.C. 102

Claims 2, 3, 11, 12, 55-57, 65, and 66 are rejected under 35 U.S.C. 102 (b), as being anticipated by Vidal et al. Applicants respectfully disagree for the reasons that follow.

Applicants have canceled claim 3 without prejudice and amended claims 65 and 66.

Vidal et al. teach a so-called "reverse yeast two-hybrid system," in that activation of the readout system is lethal (as compared to required in the conventional two-hybrid system) for the host cell harboring such a system. In that system, interaction between a bait / DNA-binding domain hybrid and a fish / transcription activation domain hybrid activates a readout system, which encodes a counter-selectable marker (such as the URA3 gene). Therefore, host cells harboring such a system can only survive in the selective medium when the bait and fish hybrids no longer interact with each other. Therefore, contrary to the Examiner's suggestion that the Vidal system is useful for identification of interacting molecules, the Vidal system is best suited for identifying "dissociator molecules," or mutations in either the bait or the fish protein that can disrupt the bait-fish interaction (Figure 1 of Vidal et al.).

Amended method claim 2 and its dependent claims are directed to identification of interacting molecules, which method employs means directed to overcome at least some of the problems encountered in traditional two-hybrid screens. Specifically, the claimed methods employ a pre-selection step (B) to eliminate false positives before screening for interacting molecules in subsequent steps. Vidal et al. neither teach nor suggest such a step.

For example, step (A) of both claim 2 and 3 provides at least one sets of host cells, each containing a different selectable marker associated with a genetic element. Therefore, the fish and bait constructs (genetic elements) are initially kept separate, e.g. inside two different sets of host

cells, or by transfecting the second plasmid into host cells only after the pre-selection step. Each set of host cells also contains a readout system that is activated upon the presence of a) either truly interacting bait and fish molecules, or b) auto-activating bait or fish molecules. Thus in step (B), those auto-activating molecules encoded by the corresponding genetic elements will activate the readout system, leading either to the killing of host cells (claim 1) or generating a visual differentiation between host cells with and without activated readout systems (claim 2). Only those pre-selected, and thus false-positive-free host cells, are then used in the remaining steps so that true positives can be identified in step (G).

On the contrary, Vidal et al. neither teach nor suggest such a pre-selection step, nor do they teach or suggest that the potentially interacting partners are initially kept separate. In fact, in the Vidal system, there is no disclosed use for a pre-selection step, and the fish and bait constructs (genetic elements) are always in the same host cells. In addition, if either one of the genetic elements is absent, it cannot serve the purpose of identifying which particular mutation or what dissociator molecule can disrupt bait-fish interaction. Accordingly, Vidal et al. fail to meet all the elements of the claimed methods.

The Office Action also alleges that "the readout system is visually toxic to the host cells as shown by growth inhibition upon activation as noted on page 10316, first paragraph of the RESULTS section which also anticipates this step in claim 3, step (B)." It is not clear as to the meaning of "visually toxic." Applicants have cancelled claim 3. However, if it is meant to support the notion that Vidal et al. also anticipates the "visual differentiation" pre-selection in claim 2, step (B), Applicants submit that such allegation is unfounded.

According to the instant claims, visual pre-selection can be used in pre-selection to eliminate false positives which auto-activate the readout system in step (B). To illustrate, if the readout system contains the *lacZ*-gene, which encodes an enzyme that can convert a colorless chemical (X-gal) in the media into a blue product, so that host cells containing an activated readout system (and, therefore, *lacZ*-gene) will turn from white to blue in the presence of X-gal. Such visual differences between cells with an activated readout system (blue cells) and cells with an non-activated readout system (white cells) can serve as a means to eliminate false positives (see Figure 20 and the paragraph bridging pages 23 and 24 of the specification). However, the first

paragraph of the RESULTS section (page 10316) in the Vidal reference fails to teach or suggest such a visual differentiation mechanism. Instead, it described the general mechanism of the reverse two-hybrid concept, which does not include the visual pre-selection step of the instant claims.

Furthermore, the word "differentiate" implies that there are two types of cells with different properties. In Vidal's case, visual inspection identifies only one type of cell - cells that grow. Hence, it is not a "differentiation" but rather "selection" of cells that grow.

The Office action also alleges that Vidal et al. used a regular grid pattern of cells as shown in page 10317, Figure 2. This objection is rendered moot as Applicants have removed claim 3 from consideration.

To anticipate a claim, the prior art reference must disclose each and every aspect of the claimed invention. Vidal et al. do not teach or suggest any of the pre-selection practices, including the use of visual means to differentiate false positives from others. Neither do they teach or suggest a method for identifying interacting molecules in non-yeast cells. Therefore, Applicants assert that Vidal et al. do not anticipate any of the claims of the instant invention. Reconsideration and withdrawal of the rejection under 35 U.S.C. 102 (b) are respectfully requested.

Claim 65 is rejected under 35 U.S.C. 102 (b) and (e) as being anticipated by Fields et al. Accordingly, Applicants have amended claim 65 to obviate this rejection.

Amended claim 65 and its dependent claims require at least one visual means for differentiating different activation states of the readout systems in host cells. Fields does not teach or suggest such visual means, especially the digital means of claim 83. Thus, the cited reference does not teach or suggest each and every limitation of the claimed invention, and cannot anticipate the amended claim 65 and its dependent claims. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim rejections under 35 U.S.C. 103 (a)

Claims 63-66 are rejected under 35 U.S.C. 103 (a), as being unpatentable over Fields et al. (U.S. Pat. No. 5,283,173).

As to the rejection of claims 63-64, Applicants respectfully disagree for the reasons which follow.

Pursuant to MPEP 2143, "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations."

First of all, Applicants submit that Fields et al. do not teach or suggest a "kit comprising host cells with a readout system which allows host cells to be counter-selected against auto-activation of said readout system." In fact, the Office Action offers no reason or argument towards the rejection of claims 63-64 at all. Applicants have been unable to identify any basis for the assertion that the Fields patent teaches or suggests the use of counter-selectable markers, nor the concept of pre-selection using selectable markers to eliminate false positives. Clarification is respectfully requested. Thus, Applicants submit that the reference does not teach or suggest all the limitations of the instant invention.

In addition, as mentioned above, the Office Action offers no motivation for arriving at the present invention or any suggestion as to how a skilled artisan could have a reasonable expectation of success in view of the Fields patent.

As to the rejection of claims 65-66, Applicants have amended claim 65 and added new claim 83 to obviate this rejection. Support for this amendment can be found throughout the specification and in the originally filed claim 57.

Amended claim 65 and its dependent claims require at least one visual means for differentiating different activation states of the readout systems in host cells. Fields does not teach or suggest such visual means, especially the digital means of claim 83. In addition, there is no motivation to combine Fields with any other cited prior art to reach the claimed kits. And it necessarily follows that a skilled artisan will have no reasonable expectation of success in arriving at the claimed invention.

Therefore, none of the three requirements for establishing a *prima facie* case of obviousness is met, and a rejection based on 35 U.S.C. 103 (a) is improper. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims are now in condition for allowance and early notification to this effect is earnestly solicited. Any questions arising from this submission may be directed to the undersigned at (617) 951-7000.

If there are any other fees due in connection with the filing of this submission, please charge the fees to our **Deposit Account No. 18-1945.** If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit account.

Respectfully Submitted,

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